

Spectrographic estimation of Fe, Mg, Ca, Zn, Sb and Pb in blood serum

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For the determination of Ca, Mg, Zn, Fe, Sb and Pb in blood serum simultaneously, a method of direct spectrochemical analysis of serum using a synchronized high voltage copper spark technique is described, which gave sufficient sensitivity for most of the elements. The method was calibrated by synthetic standard samples avoiding errors by imitating the organic contents of the serum with a condensation product of glucose urica and glycine. The analysis was carried out on human serum for the determination of the elements except Pb and Sb which were beyond our detection limit. These two elements were determined in two kinds of experimental animals which were previously injected by salts of the elements.

1. INTRODUCTION

The particular advantages of the analytical spectrographic methods, in general, are speed, remarkable sensitivity, precision, unequivocal identification. An important field, where samples of limited size occur, is the field of biological research. Organs, tissues and body fluids are analysed for various metals either to establish limits of normal distribution to ascertain departures from them under pathologic conditions, or to obtain information of diagnostic value. Many of the mineral elements found in blood are essential to health, and the knowledge of their concentration is very important (Comar & Bronner 1968). The chief role of calcium is the blood clotting, essential for contractility of involuntary muscles, acting antagonistically to sodium and potassium, formation of bones and teeth, influence on excitability of nerve fibres and centres. Under normal conditions the magnesium intake is always adequate, so that effects comparable to calcium deficiency are not seen. The effects of magnesium deficiency are severe if the calcium intake is increased. Other observations also suggest an interrelationship between calcium and magnesium. Deficiency of iron which is a part of the haemoglobin molecule, results in anaemia. Also, iron is an important intercellular catalyst of oxidation-reduction i.e., in oxidases.

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were carefully selected free from interferences and coincidence. The lines were densitometered without background correction. The transmittances obtained were converted to relative intensities using the emulsion calibration curve of the photographic plate. Intensity ratios were calculated for each pair of lines.

C. *Selection of the Electrode Diameter* : (Kabiél et al 1970)

The variation of the absolute intensity of the analysis lines of the analysed elements was studied using the different copper electrodes of diameters varying from 2 to 7 mms. For every diameter, sixteen replicates—using the same standard solution—were prepared. Results obtained for the intensity (I) of the lines together with the corresponding coefficient of variance ($C.V.$) are given in table 2 and illustrated in figure 1. From the table, it is remarkable that the electrode diameter plays an important role with respect to absolute intensity and the standard mean deviation in percent. Best results were obtained using the most wide electrode as this latter will cause less mechanical loss of the solution due to sputtering when loading the electrodes.

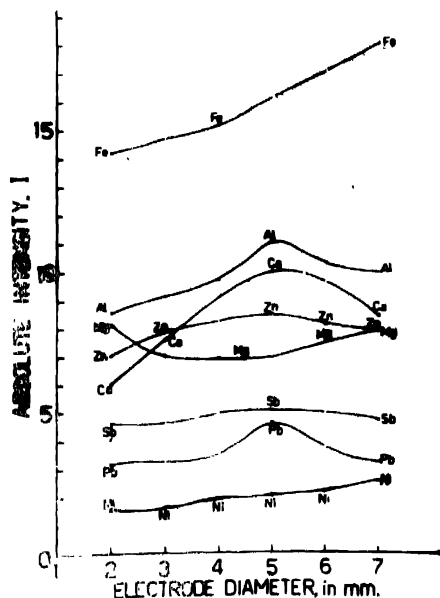


Fig. 1. Variation of the intensity with the electrode diameter.

D. *Sparking Conditions*

Excitation in the spark is largely dependent on the spark power. If no losses occur, the power in KVA is computed from the equation (Nachtrieb);

$$P = CV_0^2 f$$

where C is the capacitance in Farads, V_0 is the initial potentials in volts and f is the frequency in c/s.

Various sparking conditions of excitation concerning the initial sparking voltage, capacitance and inductance were tried to obtain a high reproducibility and reasonable accuracy of the analysis. Investigations showed that the intensity for all lines was greatly enhanced and became suitable to be measured using the following conditions :

Initial sparking voltage	17 K
Capacitance	6 nf
Inductance	0.3 mh

with such a somewhat high inductance, the spectrum was similar to an arc one which was preferred to brighten the spectrum background and obtain better emission lines of the sought elements.

E. Volatilisation Studies

Extensive moving-plate studies with one of the standards were tried. It was found, as it appears from figure 2, that each element has a characteristic manner of volatilisation with a peak emission different from the others, and

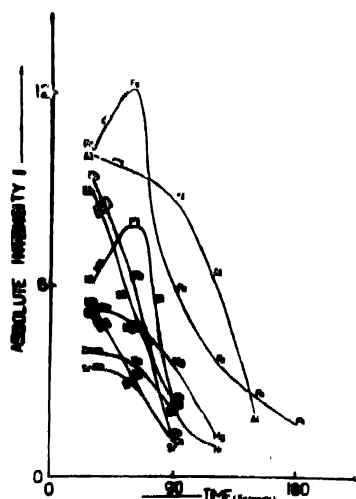


Fig. 2. Intensity-time curves for Mg, Ca, Fe, Zn, Al, Ni, Sb and Pb.

that all the elements are volatilised at the first 100 secs. of exposure with remarkable sharp decrease in their corresponding line intensity for more than 120 secs. accompanied by an increase in the darkness of the background. Accordingly, 100 secs were selected to be the suitable time of excitation.

Table 2. Effect of electrode diameter on absolute intensity and coefficient of variance of the spectral lines of the sought elements.

Electrode diameter	Ca		Fe		Ni		Pb		Al		Sb		Mg		Zn	
	I	C.V	I	C.V	I	C.V	I	C.V	I	C.V	I	C.V	I	C.V	I	C.V
2	6.03	10.4	14.25	12.1	1.56	8.4	3.17	13.3	8.58	7.2	4.61	9.6	8.15	14.9	7.02	11.1
3	7.64	10.1	14.82	12.0	1.61	7.9	3.29	6.1	9.20	8.2	4.64	8.6	7.01	13.2	7.92	10.1
4	9.20	7.8	15.25	8.2	1.90	5.1	3.52	11.2	9.27	5.1	5.02	8.8	7.00	9.1	8.70	6.1
5	10.10	3.1	16.35	4.1	2.05	2.3	4.66	6.3	11.20	3.1	5.11	4.6	7.04	5.3	8.58	3.9
6	9.75	3.3	17.25	3.1	2.22	1.9	3.87	5.1	10.40	0.2	5.01	4.5	7.60	1.2	8.21	1.2
7	8.58	1.2	18.20	1.8	2.61	1.1	3.27	2.6	10.10	1.6	4.08	2.1	8.00	1.2	8.00	1.1

3. APPLICATION

i) Human Serum

The blood was drawn from the intercubital fossa vein, in the arm, using a very clean and dry 20 mm hypodermic syringe fitted with special Pt-Ru alloy needle. The blood was then transferred immediately to a very clean centrifuge polyethylene bottle, noting that the blood must run on the wall of the tube to avoid any sort of haemolysis. The blood was centrifuged at $3000 \times G$ for 15 minutes. The serum fraction was withdrawn with a similar hypodermic syringe. Every one ml of serum was mixed with one ml of solution containing Ni and Al as internal standards, and the resulting solution was shaken by hand for complete mixing. Then 0.2 ml of this solution was transferred on the clean electrode top, treated as the standards and tested spectrographically. Using the constructed working curves, shown in figure 3, the concentrations of the sought elements were determined. Results obtained are given in table 3.

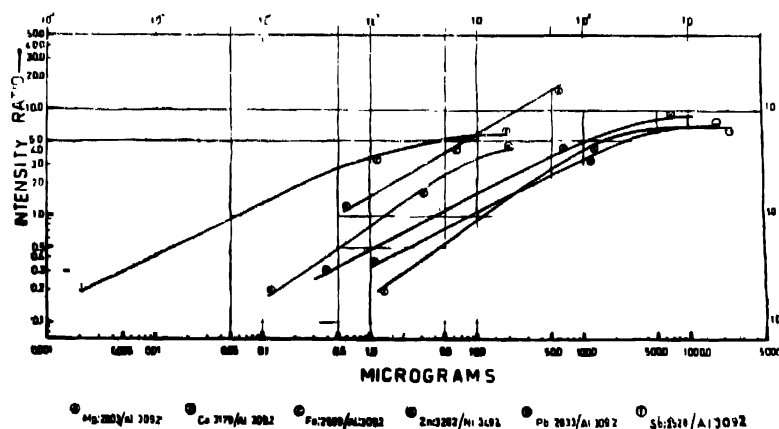


Fig. 3. Analytical copper spark working curves for Mg, Ca, Fe, Zn, Pb and Sb.

Table 3. Concentration range and average values obtained for normal human serum, carried on a group of 29 volunteers.

Element	Concentration range in γ/ml	Average concentration in γ/ml
Fe	0.9— 1.3	1.15
Mg	17 — 35	24
Ca	90 —110	105
Zn	0.6— 2.1	1.3
Sb	N.D.	N.D.
Pb	N.D.	N.D.

ii) *Animal Serum*

The determination of the elements sought in the serum of two experimental animals, was performed. As Pb and Sb are not normally present in normal blood, we performed some injection treatments with solutions containing Pb and Sb.

a) *Estimation of Pb* : A group of six rabbits was selected, and 5 mls of blood from each was withdrawn. The serum was obtained as described before, treated in the same way and tested spectrographically. Results obtained are given in table 4. Five mls. of lead sub-acetate were then injected in the marginal vein of each rabbit. After 24 hours, again 5 mls of blood were withdrawn, serum obtained, treated as previously explained and tested spectrographically. Results obtained are given in table 4.

Table 4. Average concentration of the elements sought before and after injection with lead subacetate—in group of 6 rabbits.

Element	Average concentration before injection in γ/ml	Average concentration after injection in γ/ml
Fe	1.41	0.99
Mg	22.40	19.00
Ca	136.60	210.00
Zn	1.69	1.11
Sb	N.D.	N.D.
Pb	N.D.	1.5

b) *Estimation of Sb* : Two mls of blood, withdrawn from the tail of a group of six rats were treated as described before and the serum was tested spectrographically. Results obtained are given in table 5. Two mls of Tartaremetic were injected through the large vein in the tail of each rat. After 24 hours 2 mls of blood were withdrawn, serum obtained and tested spectrographically. Results are given in table 5.

Table 5. Average concentration of the sought elements, before and after injection with Tartaremetic—in a group of 6 rats.

Element	Average concentration before injection in γ/ml	Average concentration after injection in γ/ml
Fe	1.10	1.5
Mg	15.10	13.2
Ca	119.20	125.2
Zn	0.70	N.D.
Sb	N.D.	3.5
Pb	N.D.	N.D.

4. DISCUSSION

Accuracy is one of the broad objects of spectrochemical analysis where improvement by any order of magnitude would result in vast new fields of application. In biology and medicine better accuracy, could result in a new diagnostic tool. Accuracy in quantitative spectrographic work depends on several factors such as the source of excitation, sort and shape of electrodes and the photometric calculations.

To attain best accuracy, the spark excitation source was used which had proved, in routine quantitative work, to be most suitable compared with the d.c. arc. Generally, spark has relatively poor concentrational sensitivity, primarily because of relatively high background radiation. The use of high voltage and capacitance, in order to reach maximum sensitivity, had provided us with a spectra which were arc-like. On the otherhand, the use of a somewhat relatively high inductance, was purposely chosen in order to brighten the arc-like spectra background.

A course approaching high accuracy and in certain cases surpassing the sensitivity of the d.c. arc, is the copper spark electrode technique. A comparison between excitation of both carbon and copper electrodes was done. It was found that, under strictly selected excitation conditions, both electrodes yielded high sensitivity and relatively good precision, but that of copper was more high in sensitivity. It was also found that the diameter of the electrodes played an important role with sensitivity. It was observed that the sensitivity of the determinations increased as the electrode diameter increased, owing to the fact that the sample loss from the electrode due to the sputtering was less.

The measurement of the intensity of the spectral lines using the microphotometer, was accurately done. The shoulder, of the working curves, at low transmittance values which resulted partially from the scattered light that entered the microphotometer slit from portions of the spectrograms around the line itself, had been somewhat corrected by measuring 2/3 of the magnified line.

For internal standardisation, different elements were selected such as Al, Ni, and Mn. Unfortunately, it was found that the quantity of Mn added as internal standards was somewhat small with respect to the amount already present in blood which might introduce a source of error during the application of the method. However, Al and Ni were tested, and the standard mean deviation for each sought element with respect to both Al and Ni with and without B.G. correction was calculated. Results are given in table 6.

Finally, we have to state clearly that we had not strictly used the control lines (Rais Ultimas) of the analysed elements. It was found by Nachtries and also was observed by us (Kabel *et al* 1970) that other lines than Rais Ultimas

became more enhanced using the copper spark technique and was found to be satisfactory to make use of especially at low concentrations.

Table 6. The standard mean duration in percent (c.v.) calculated for each analysed element with respect to Al and Ni. Results are mean of 8 determinations.

Analysed element	Ni		Ni	
	Without B.G. correction	With B.G. correction	Without B.G. correction	With B.G. correction
Fe	4.4	6.6	5.8	9.9
Ca	6.3	8.4	9.6	10.3
Mg	5.4	8.1	6.8	7.7
Zn	9.4	10.1	6.3	8.5
Pb	8.7	10.1	9.1	9.9
Sb	8.6	11.9	9.3	11.2

5. CONCLUSION

It is seen from the previous studies that there is an improvement in the rapidity of the determination and also much accuracy and sensitivity. Also, the validity and superiority of the proposed method for the estimation of micrograms of Zn, Fe, Ca, Mg, Pb and Sb in biological fluids can be easily ascertained.

The results obtained are extraordinary sensitive and exactly similar to that in literature.

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